Short Hydrogen Bonds, Circular Dichroism, and Over-Estimates of Peptide Helicity**

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Short polypeptide fragments from proteins were once believed to be unstructured. The observation of structure in the C peptide (connecting peptide) from the N terminus of ribonuclease A has spawned the widespread study of the structure of peptides in solution.[1] Their structure is often characterized by using circular dichroism (CD) spectroscopy, in part because helical peptides have a distinctive CD spectrum, with a positive peak at 190 nm, and negative peaks at 208 nm and 220 nm (or 222 nm). As discussed recently,[2] the average fractional helicity, f_H, of an N-residue peptide may be related to the observed mean residue molar ellipticity at 220 nm, $[\theta]_{220}$, by Equation (1).

$$f_H = \frac{[\theta]_{220}}{[\theta_{H\infty}]_{220}\left(1 - \frac{k}{N}\right)} \quad (1)$$

$[\theta_{H\infty}]_{220}$ is the ellipticity of an infinitely long, completely helical peptide and k is an end-effect correction, the value of which is ~ 3. Estimated values for $[\theta_{H\infty}]_{220}$ range from $-37\,000$[3] to $-44\,000$ deg cm^2 d mol^{-1}.[4] The recent report[2] of peptides of the type AcHel-(Ala$_4$Lys)$_n$Ala$_2$-NH$_2$ exhibiting values of $[\theta]_{220}$ more negative than $-44\,000$ and as low as $-55\,000$ deg cm^2 d mol^{-1} is thus quite startling, and has obvious implications concerning the application of Equation (1) to interpret CD measurements. Herein, we rationalize the observation of unprecedented ellipticity at 220 nm by using recently improved theoretical methods for calculating the circular dichroism of polypeptides.

In the discussion of their observation of unprecedented intensity at 220 nm, Kemp and co-workers remarked that the nπ* band at 220 nm has yet to be satisfactorily modeled by theory.[2] Although this comment was correct at the time, the situation has changed. We have used ab initio calculations on *N*-methylacetamide in a continuum model of solvent to realize an improved description of the electronic states and transitions of the amide chromophore in proteins.[5] Using a set of parameters derived from the ab initio calculations, the matrix method for calculation of the CD of polypeptides,[6] and coordinates from X-ray structures, we have been able to reproduce $[\theta]_{220}$ for a set of 29 proteins with a correlation of ~ 0.9.[7] Comparable improvements have also been achieved using semiempirical parameters.[8] Our calculations consider only the nπ* and $\pi\pi$* transitions of the amide backbone chromophore. Contributions from higher-energy transitions are ignored, as are all transitions from side-chain chromophores or disulfide bridges. Whilst the calculations are not fully quantitative and tend to underestimate $[\theta]_{220}$ by about 10%, they are sufficiently accurate to explore semiquantitatively the dependence of $[\theta]_{220}$ on the precise geometry of the residues in a helical peptide.

Two potentially important features of the experimental study by Kemp and co-workers are the use of ethylene glycol as a co-solvent and a variation in temperature, which can fall as low as 253 K.[2] Ab initio calculations have suggested that ethylene glycol promotes the formation of strong (and short) hydrogen bonds.[9] Detailed modeling of possible conformations of AcHel-(Ala$_4$Lys)$_n$Ala$_2$-NH$_2$ in mixed solvents is beyond the scope of this report. However, we present calculated CD spectra for a range of helical conformations of a model polyalanine peptide, which suggest that the observed anomalous CD may be because of the formation of short hydrogen bonds.

Spectroscopic calculations may be quite sensitive to the precise conformation of the peptide chain. Therefore, it is important to prepare representative structures carefully. The macromolecular modeling software CHARMM was used.[10] Minimizations of the blocked peptide, Amn-Ala$_{20}$-Cbx, were performed within the generalized Born continuum solvent model, with parameters appropriate for water.[11] We have examined typical geometries for the α, 3_{10}, and π helices. Peptides with α-helical ($\phi = -48^\circ$, $\psi = -57^\circ$; and $\phi = -63^\circ$, $\psi = -41^\circ$), 3_{10}-helical ($\phi = -60^\circ$, $\psi = -30^\circ$), and π-helical ($\phi = -57^\circ$, $\psi = -70^\circ$) conformations were built for a range of main-chain hydrogen-bond lengths from $d_{O-N} = 2.5$ Å to $d_{O-N} = 3.8$ Å (d_{O-N} is the oxygen–nitrogen separation) in increments of 0.1 Å. Two α-helical geometries were examined. One, with dihedral angles (-48°, -57°), is the classical Pauling–Corey helix; the second has dihedral angles (-63°, -41°) close to the mean values observed in helices in proteins.[12]

To generate the desired geometry, constraint potentials were used for both the dihedral angles and the hydrogen-bond distances. Force constants for the constraints with values of 500, 1000, and 2000 kcal mol^{-1} Å^{-2} (kcal mol^{-1} rad^{-2} for the angle constraints) were explored. We identified the values that gave structures in which the deviation from the desired dihedral angles was within 1° and hydrogen-bond distances within 0.1 Å. We chose the values that met the criteria across the range of hydrogen-bond distances and led to conformations with the lowest energy.

The peptide studied by Kemp and co-workers probably adopts an α-helical conformation. Figure 1 shows that first principles CD calculations predict a marked linear relationship between hydrogen-bond length and $[\theta]_{220}$, the intensity at 220 nm. For completeness, we have also investigated 3_{10} and π helices. They show trends similar, if less pronounced, to the α helix. A very intense band at 220 nm suggests the presence of short hydrogen bonds. Shortening from a conventional oxygen–nitrogen separation of about 3.0 Å to 2.8 Å or 2.7 Å is predicted to lead to a sizable enhancement of the intensity at 220 nm.

From the potential-energy surfaces calculated using constrained minimization within the generalized Born continuum solvent model (Figure 2) and the corresponding Boltzmann populations, the changes in temperature in the experimental system from 333 K to 253 K cannot entirely account for the

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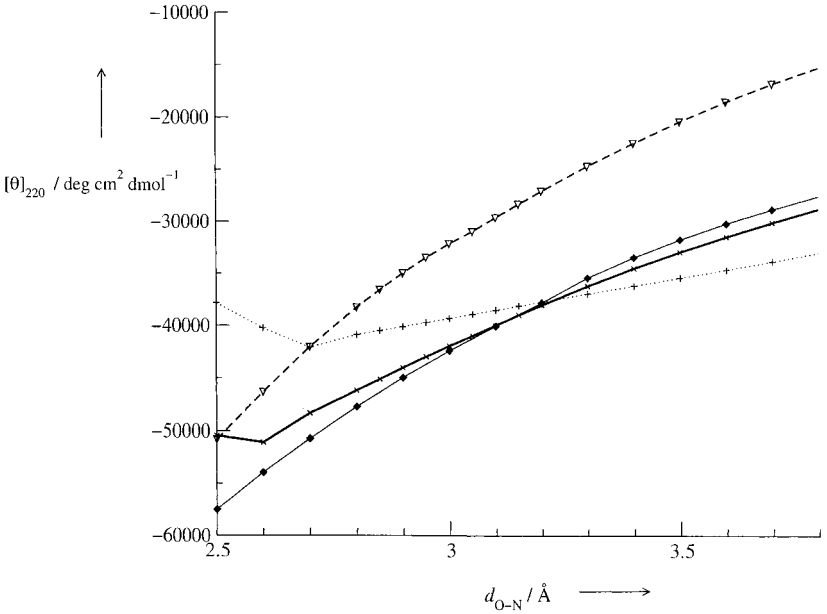

Figure 1. Mean residue ellipticity at 220 nm, $[\theta]_{220}$, calculated as a function of main-chain hydrogen-bond length for different helices, d_{O-N}: ($\triangledown$) = 3_{10} helix ($-60°$, $-30°$); (+) = π helix ($-57°$, $-70°$); ($\times$) = α helix ($-48°$, $-57°$); ($\blacklozenge$) = α helix ($-63°$, $-41°$).

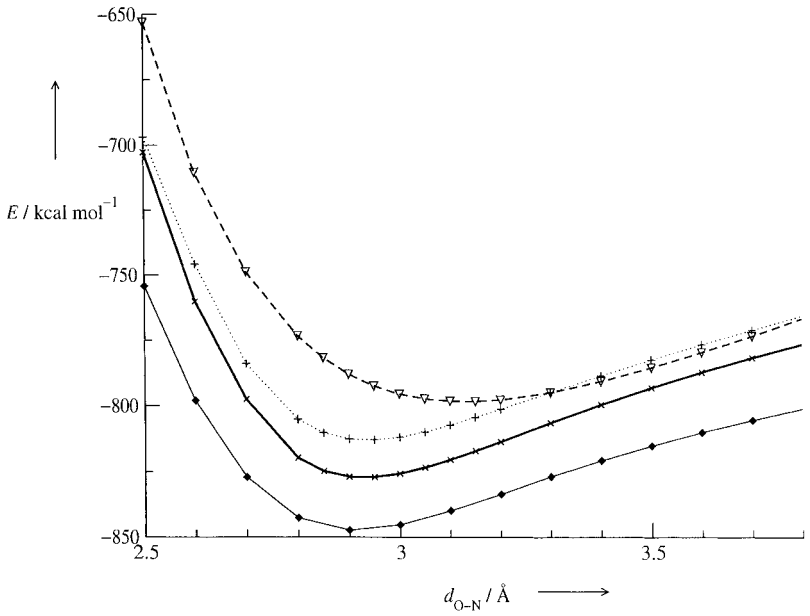

Figure 2. The potential energy, E, calculated as a function of main-chain hydrogen-bond length for different helices in water, d_{O-N}: ($\triangledown$) = 3_{10} helix ($-60°$, $-30°$); (+) = π helix ($-57°$, $-70°$); ($\times$) = α helix ($-48°$, $-57°$); ($\blacklozenge$) = α helix ($-63°$, $-41°$).

large changes in $[\theta]_{220}$. However, an explanation is suggested by ab initio calculations on the solvent influence on peptide hydrogen-bond stability in water, ethanol, ethylene glycol, and trifluoroethanol.[9] This investigation of hydrogen-bond strength with the interaction with one or more solvent molecules found a significant cooperative effect strengthening the hydrogen bond in the presence of certain high-polarity solvent molecules, which selectively bind to the helical conformation of peptides.

In a helical peptide, solvent molecules can hydrogen bond to unsatisfied hydrogen-bonding valences at the termini of the helix. Hydrogen-bonding valences within the core are satisfied by main-chain hydrogen bonds. Solvent molecules may be able to hydrogen bond directly to core amide residues. However, even if they do not, recent theoretical work[13] suggests that there is a high degree of cooperativity in chains of hydrogen-bonded amides, which may provide a mechanism for propagating short hydrogen bonds along a helix.

Thus, calculations of the CD of helical peptides from first principles indicate that $[\theta]_{220}$ is sensitive to the main-chain hydrogen-bond length. Changes in the hydrogen-bond lengths alter the distances, orientation, and interactions between the electronic excited states on different amides. Detailed analysis of our matrix-method calculations suggests that these interactions and their variation with hydrogen-bond length are quite sensitive to the particular geometry of the peptide chain. In the Pauling–Corey helices, the predominant effect of a shorter hydrogen bond is to increase the interaction between the $\pi\pi^*$ electric transition dipole moments of the two hydrogen-bonded peptide groups. In the other α-helical geometry that we examined, shorter hydrogen-bond lengths increase the interaction between the $n\pi^*$ transition on one amide group with the $\pi\pi^*$ transition on the subsequent amide along the chain. Whilst the change in the intensity at 220 nm is the most striking feature of the experimental data, there are also significant changes at 208 nm. The precise geometry of the peptide is unknown and it may be that both mechanisms play a role in modifying the CD spectrum.

Ab initio calculations provide strong evidence that ethylene glycol promotes short hydrogen bonds. Thus, we propose that the unprecedented intensity observed by Kemp and co-workers for a helical peptide in a water/ethylene glycol solvent is a result of short hydrogen bonds. Similar observations of intense CD in trifluoroethanol/water and in aqueous salt (NaCl) solutions have also been reported.[2] Short hydrogen bonds may be significant in the former; the effect of salt is perhaps less clear. The short hydrogen-bond hypothesis might be tested experimentally through NMR spectroscopy measurements or theoretically by molecular dynamics simulations of the peptide in explicit solvent. In the latter case, however, a typical potential based on pair-wise additive interactions may not be adequate, as noted by Guo and Karplus.[9]

Our calculations demonstrate that short hydrogen bonds will influence the CD spectra of peptides. They present one plausible explanation for the origin of anomalously high ellipticity. Other factors may also be important. Woody has suggested[14] that the interaction of the charged lysine residues

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with amide carbonyl oxygen atoms is likely to lead to strong mixing of the $n\pi^*$ and $\pi\pi^*$ transitions in those amide groups.

In conclusion, some helical structures formed by simple polypeptides in solution may have unusually short main-chain hydrogen bonds. Our calculations indicate that the helicity of such peptides will be over-estimated by CD analysis based on literature calibration of helical content. Whilst much is still to be established, if short hydrogen bonds can form under certain conditions, the use of CD to estimate helicity will have to be complemented by another experimental probe that is sensitive to hydrogen-bond length.

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Orientation Dependence of Energy Transfer in an Anthracene – Porphyrin Donor – Acceptor System**

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The rates of electronic energy transfer (EET) reactions mediated by both dipole – dipole and electron-exchange mechanisms have long been postulated to be critically dependent, amongst other things, on the distance and mutual orientation between the donor and acceptor moieties.[1, 2] Recent experimental validation of these theoretical predictions comes from energy-transfer studies carried out with rationally designed, photochemically active donor – acceptor (D – A) assemblies.[3] Amongst such D – A systems, those based on the porphyrinoid class of chromophores are of relevance to the present work. A number of studies carried out with the covalently/noncovalently linked porphyrin – porphyrin[3b,c, 4] and porphyrin – nonporphyrinic chromophore[5] assemblies have dealt with the effect of D – A distance on the rates of EET reactions but, relatively less attention has been paid to the corresponding orientation effects. In addition, to our knowledge, orientation dependence of EET has not been unequivocally demonstrated in a porphyrin-based system where the donor and the acceptor subunits are disposed at two distinctly different orientations in a given D – A ensemble. Here, we demonstrate the orientation dependence of energy transfer in a simple, porphyrin-based, D – A system **3** where the donor anthracene subunits are linked both at the axial and peripheral sites of a tin(IV) porphyrin scaffold, Scheme 1. Fluorescence-emission and excitation spectra reveal that light absorbed by the "peripheral" anthracene unit of **3** is efficiently transferred to the porphyrin but, that absorbed by the "axial" anthracene subunits is not.

The syntheses of **3** and the corresponding "reference" compounds **1** (where the anthracene subunit occupies only a peripheral position) and **2** (where the anthracene subunits occupy only the axial sites) are illustrated in Scheme 1. These new porphyrins have been characterized by elemental analysis, UV/Vis, ^{1}H and ^{13}C NMR spectroscopy, and electrochemical methods. In the ^{1}H NMR spectra, the spacer methylene protons connecting the porphyrin and anthracene chromophores of **1** resonate at $\delta = 6.33$, whereas the corresponding axial methylene protons of **2** resonate at $\delta = 5.65$ a result of the ring-current effect exerted by the basal porphyrin macrocycle. In the spectrum of **3**, resonances arising from the two peripheral and the four axial methylene protons are at $\delta = 6.32$ and 5.62, respectively. The UV/Vis spectra of **1**, **2** and **3** (in CH_2Cl_2) are nearly equivalent to the summation of the

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